REMARKS

Claims 1-9 are pending in this application. Claims 4-7 stand rejected under 35 U.S.C. § 101 as being directed to non-statutory subject matter. Claims 1-9 stand rejected under 35 U.S.C. § 112, second paragraph for indefiniteness. Claims 1-9 also stand rejected under 35 U.S.C. § 102 for anticipation. Each of these rejections is addressed below.

Amendments

Claims 1-9 have been amended to clarify the subject matter originally claimed. In particular, the terms "viral protein" and "viral structure protein" in claim 2 were unified into "viral protein." Support for this amendment can be found throughout the specification, for example, at page 7, lines 1-4. Support for the amendment to claim 1 is found, for example, at page 7, lines 1-21, page 9, lines 4-29, and page 10, line 5 to page 15, line 20. Support for the amendment to claim 3 is found, for example, at page 7, lines 29-31, page 9, lines 4-9, page 11, lines 10-13, and in Figure 1. Support for the amendment to claim 4 is found, for example, at page 8, line 25 to page 9, line 3, and page 9, lines 12-14. Support for the amendment to claim 5 is found, for example, at page 8, line 25 to page 9, line 3, page 9, lines 12-14, and page 11, lines 5-9. Support for the amendment to claim 6 is found, for example, at page 7, lines 1-21, page 8, line 25 to page 9, line 9, and page 10, line 5 to page 15, line 20. Support for the amendment to claim 7 is found, for example, at page 7, lines 29-31, page 8, line 25 to page 9, line 9, page 11, lines 10-13, and in Figure 1. Support for the amendment to claim 9 is found, for example, at page 8, line 25 to page 9, line 3. Support for new claims 10-18 is found in the Examples and Detailed Description of the Invention. Support for new claims 19-22 is found at page 3, lines 32-39, and page 11, line 24 to page 12, line 25. No new matter is introduced by these amendments.

A "marked up" version of the specification and claims showing the changes made and an appendix of clean versions of the added and replacement paragraphs and of the

claims as pending is attached.

Priority Document

A certified copy of the Japanese priority document, JP 2000/152726 filed May 18, 2000, referred to on the transmittal sheet of the above-referenced application is enclosed herewith as required by 35 U.S.C. § 119(b).

Sequence Listing

An amended Sequence Listing, including the sequences described on page 14, lines 29 and 31, satisfying the requirements of 37 C.F.R. §§ 1.821-1.825 is enclosed herewith, and the objection raised in the Office Action relating to the sequence rules may be withdrawn.

Rejection Under 35 U.S.C. § 101

Claims 4-7 stand rejected under 35 U.S.C. § 101 on the ground that the claimed invention is directed to non-statutory subject matter. As recommended by the Office, Applicants have amended the rejected claims to recite "isolated DNA." In view of this amendment, this basis for the rejection may be withdrawn.

Rejections Under 35 U.S.C. § 112, second paragraph

Claims 1-9 stand rejected, under 35 U.S.C. § 112, second paragraph, as being indefinite.

In particular, claims 1–9 were deemed unclear because claims 1 and 5 state that the foreign gene is located "downstream" of genes encoding viral proteins.
The current clarifying amendment has met this basis for the rejection. By this amendment, Applicants have specified that a foreign gene is "located downstream of **a** gene encoding **a** viral protein in the negative strand genomic RNA contained within said vector" (emphasis added) and not necessarily downstream of **all** viral genes (*i.e.*, at the 5'

terminus, after the L gene). In other words, the foreign gene is neither at the 3' end of the genome nor inserted in the middle of any viral gene. Rather, the foreign gene is positioned downstream of at least one gene (*e.g.*, inserted after the first, second, third, fourth, fifth, or sixth viral gene). Applicants submit that this amendment eliminates any discrepancy between claims 1 and 5 and claims 2 and 6. Accordingly, reconsideration and withdrawal of the rejection is requested.

In addition, claims 2 and 6 were deemed vague and indefinite because paragraph (f) states that the foreign gene is inserted between the 6^{th} viral protein gene and the 3' end of the RNA yet the viral genome is 3' to 5'. Applicants submit that option (f) as originally drafted is sufficiently clear, in that the foreign gene is "inserted between the 6^{th} gene [counting from the 3' end of the negative strand genomic RNA] encoding a viral protein" and "the trailer sequence." However, to expedite prosecution, the term "trailer sequence" has been replaced with the term "the 5' end of said negative strand genomic RNA". Applicants submit that this amendment eliminates any potential confusion and, accordingly, request reconsideration and withdrawal of the rejection.

A further basis for this rejection states that the claims 3 and 7 are indefinite for reciting the phrase "in their order." Applicants have amended claims 3 and 7 to require that the 1^{st} to 6^{th} genes encoding viral proteins, counting from the 3' end to the 5' end of the negative strand genomic RNA contained within the vector, arise in the following order: NP gene, P gene, M gene, F gene, HN gene, and L gene. Applicants recognize that certain paramyxoviruses do not share this genetic order; such species would accordingly be excluded from the scope of claims 3 and 7. Applicants submit that this amendment eliminates any confusion and, accordingly, request reconsideration and withdrawal of the rejection.

Finally, the Office rejects claims 4 and 5 for respectively reciting the phrases "or their complementary strands" and "its complementary strand." This basis for the rejection has been overcome by the present amendment. Specifically, claims 4 and 5 have respectively been amended to recite an isolated DNA corresponding to either "the negative strand genomic RNA contained in a replicable paramyxovirus vector" or "the complementary RNA of said negative strand genomic RNA". Applicants submit that these amendments eliminate any confusion and, accordingly, request reconsideration and withdrawal of the rejection.

Rejections Under 35 U.S.C.§ 102

Claims 1-9 stand rejected, under 35 U.S.C. § 102(a), as being anticipated by either Tokusumi *et al.* (*The Third Annual Meeting of the American Society of Gene Therapy*, Program 890 (May 31-June 4, 2000)) and Kato *et al.* (*Journal of Virology* 73:9237-9246 Claims 1-9 stand rejected, under 35 U.S.C. § 102(b) and 102(e), as being respectively anticipated by Conzelmann *et al.* (EP 0 702 085 A1) and Conzelmann *et al.* (US Pat. No. 6,033,886).

The Tokusumi Reference

Claims 1-9 stand rejected, under 35 U.S.C. § 102(a), as being anticipated by Tokusumi *et al.* This rejection is respectfully traversed.

The Tokusumi reference is not prior art to the present invention. This reference was published May 3, 2000[1], less than one year before the October 31, 2000 filing date of the present application. Applicants are the joint inventors of the pending claims and are the joint contributors of any relevant information in that reference, notwithstanding the inclusion of the additional authors, and a Declaration by Yoshiyuki Nagai, a co-inventor on this application, to this effect is attached. Accordingly, the Tokusumi reference does not constitute prior art to this application (*In re Katz*, 687 F.2d 450 (C.C.P.A. 1982)), and

[1] Applicants enclose, as Appendix A, a copy of a web page from the internet site of *American Society of Gene Therapy* ("AGST") indicating that accepted abstracts of the ASGT 2000 Annual Meeting were available as of May 3, 2000.

the § 102(a) rejection may be withdrawn.

The Kato Reference

Claims 1-9 stand rejected, under 35 U.S.C. § 102(a), as being anticipated by Kato *et al.* This rejection is respectfully traversed.

The Kato reference is not prior art to the present invention. This reference was published in the November 1999 issue of the *Journal of Virology*, less than one year before the October 31, 2000 filing date of the present application. Applicants are the joint inventors of the pending claims and are the joint contributors of any relevant information in that reference, notwithstanding the inclusion of the additional authors, and a Declaration by Yoshiyuki Nagai, a co-inventor on this application, to this effect is attached. Accordingly, the Kato reference does not constitute prior art to this application (*In re Katz*, 687 F.2d 450 (C.C.P.A. 1982)), and the § 102 rejection may be withdrawn.

The Conzelmann References

Claims 1-9 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Conzelmann *et al.* (EP 0 702 085 A1; herein referred to as "the Conzelmann '085 application") and under 35 U.S.C. § 102(e) as being anticipated by Conzelmann *et al.* (US 6,033,886; herein referred to as "the Conzelmann '886 patent"). Specifically, the Examiner, referring respectively to claims 1, 3-5, 8, and 9 and claims 1-4, 8, and 9 of the Conzelmann '085 application and the Conzelmann '886 patent, asserts that these references teach a replicating paramyxovirus comprising an insertion in the open reading frames of the pseudogene and M regions. These rejections are respectfully traversed.

The fundamental purpose of § 102 is to prevent the granting of a patent to an applicant on subject matter that is already within possession of the public. Thus, it follows that a prior art reference, to anticipate a claimed invention, must have placed the invention in the public domain. Anticipation under 35 U.S.C. § 102 therefore requires that the invention disclosed by the prior art reference must be identical to the claimed invention in each and every aspect. As stated in *Hybritech Inc. v. Monoclonal Antibodies,*

Inc., 802 F.2d 1367, 231 U.S.P.Q. 81 (Fed. Cir. 1986), "[I]t is axiomatic that for prior art to anticipate under 102 it has to meet every element of the claimed invention." Neither of the cited Conzelmann references meets this standard in supporting the present rejections.

The invention as now claimed requires a replicable paramyxovirus vector carrying a foreign gene that is located downstream of a gene encoding a viral protein in the negative strand genomic RNA contained with the vector, wherein the vector is capable of expressing the foreign gene. Nowhere does the Conzelmann'085 application or the Conzelmann'886 patent disclose such a replicable paramyxovirus vector.

As an initial matter, with respect to claims 1-9, the claimed replicable paramyxovirus vectors require that a foreign gene is positioned downstream of a viral gene .... in the negative strand genomic RNA. The claimed replicable paramyxovirus vectors therefore differ from a viral vector that includes an insertion in the open reading frames of the pseudogene and M regions as asserted by the Office. Accordingly, on this basis alone, the 102 rejections should be withdrawn.

Furthermore, Applicants point out that the Conzelmann references fail to teach paramyxovirus vectors that explicitly express a foreign protein from a gene that is positioned downstream of a paramyxovirus gene. Instead, the Conzelmann references describe only the insertion and expression of a foreign gene in the so-called pseudogene ("Ψ") region of the rabies virus. In contrast to the rabies virus, paramyxoviruses such as the Sendai virus do not have a pseudogene region. Given that paramyxoviruses do not possess a pseudogene, the Conzelmann references for this reason too also fail to anticipate the claimed paramyxovirus vectors.

Applicants also point out that new claims 10-18 are drawn to Sendai virus vectors. As the Conzelmann references fail to teach Sendai virus vectors as presently claimed, Applicants submit that these new claims are free of the anticipation rejection in view of the Conzelmann '085 application or Conzelmann '886 patent.

Furthermore, new claims 19-22 are directed to methods of regulating expression of a foreign gene positioned within a paramyxovirus vector. As the Conzelmann references

do not disclose methods for regulating the expression of a foreign gene based on positioning a foreign gene downstream of a paramyxovirus gene, Applicants submit that claims 19-22 are also free of the anticipation rejection based on the Conzelmann '085 application or the Conzelmann '886 patent.

Nonobviousness

Applicants further submit that the Conzelmann teachings, when taken as a whole, fail to teach or suggest the claimed replicable paramyxovirus vectors; Sendai virus vectors; or methods for regulating the expression level of a foreign gene within a replicable paramyxovirus vector. As is discussed above, the Conzelmann '085 application and the Conzelmann '886 patent are largely focused on engineering rabies virus. Given the focus on the rabies virus, it is not surprising that never once does Conzelmann indicate that a foreign gene should be placed downstream of a paramyxovirus gene or a Sendai virus as presently claimed by Applicants.

Rabies Virus and Paramyxoviruses are Biologically Dissimilar

Applicants point out that there is no reasonable scientific basis for inferring that the biology of expressing a foreign protein using a rhabdovirus such as the rabies virus reasonably extrapolates to paramyxoviruses. Rhabdoviruses and paramyxoviruses are different in their morphology, genomic structure, and properties of viral proteins. While mature particles of rhabdoviruses have a distinct bullet- or rod-like shape, the particles of a paramyxoviruses are spherical, reflecting differences in the properties of viral proteins that contribute to particle formation. Unlike rhabdoviruses, which have an envelope consisting entirely of G protein, the paramyxovirus consists of two envelope proteins, HN and F. HN and F proteins are encoded separately in the viral genomic RNA, and are functionally different, the HN contributes to adsorption onto a target cell, and F induces fusion to the cell membrane. Functional experiments have also demonstrated that the envelope proteins of the two viruses are different. For example, studies of phenotypic mixing between a paramyxovirus (Sendai virus) and an rhabdovirus (vesicular stomatitis

virus; "VSV") suggested that the Sendai virus envelope proteins are not able to compensate for G protein of VSV in budding (see, for example, Metsikko *et al.*, 1989, *J. Virol.* 63: 5111-5118; copy enclosed).

In addition, the rabies virus G-protein inserts into cellular membranes, allowing for fusion of the virus to occur. The G protein then undergoes a conformational shift under acidic conditions that stabilizes the protein, exposing a hydrophobic domain that then inserts into cellular membrane. Fusion of the rabies virus occurs in the endocytic vesicle, where there is an acidic pH. Cells that are infected with rabies virus can be recognized by the prominent cytoplasmic inclusion bodies; these are called Negri bodies. On the other hand, the paramyxovirus induces fusion at neutral pH at the cell surface.

Conzelmann himself recognized differences between rhabdoviruses and paramyxoviruses. For example, regarding the use of reverse genetics for construction of recombinant viruses, Conzelmann has stated "whereas the entire rhabdovirus life-cycle, including assembly and budding of virions, is correctly performed in the presence of vaccinia virus, Sendai paramyxovirus virion assembly is prevented by vaccinia virus," and he asserted that such peculiarities "have to be taken account in the design of reverse genetics experiments" (see, Conzelmann, 1996, *J. Gen. Virol.* 77: 381-389 at page 385, left column; copy enclosed). Furthermore, in Sendai virus, unlike rabies virus, efficient replication is observed only when the total nucleotide number of the genome consists of a multiple of six (see, Calain, P. and Roux, L., 1993, *J. Virol.* 67: 4822-4830). Based on the differences in the structure and property of the viral proteins between the two viruses, Conzelmann has also acknowledged that "differences in the assembly process apparently exist between rhabdoviruses" (see, Conzelmann, 1996, *J. Gen. Virol.* 77: 381-389 , at page 385, right column; copy enclosed) [and paramyxoviruses].

Given these aforementioned differences, it is unreasonable to assume that rhabdovirus and paramyxovirus expression systems are equivalent, and it would not have been obvious to construct the claimed paramyxovirus vectors given the Conzelmann teaching relating to engineering rabies virus. The biological principles underlying

rhadoviruses are not interchangeable with paramyxoviruses for expressing a foreign protein. As discussed above, rhabdoviruses such as rabies virus and paramyxoviruses such as Sendai virus are neither identical nor closely-related. Rhabdoviruses and paramyxoviruses are morphologically, physiologically, and genetically distinct. Given these differences, the expression of a foreign protein in a rhabdovirus such as the rabies virus and paramyxovirus such as the Sendai virus is also dissimilar. Accordingly, absent a logical predicate, it is unreasonable to assume that rabies virus and paramyxoviruses are equivalent and interchangeable expression systems.

The Conzelmann References Fail to Teach Engineering Paramyxoviruses For Foreign Gene Expression

Applicants also point out that the Conzelmann references do not teach the production of paramyxoviruses having a foreign gene downstream of a viral gene. Although the recovery rate of full-length recombinant rabies virus from cDNA is not apparent in either the Conzelmann '085 application or the Conzelmann '886 patent, there is a description concerning the efficiency of virus-rescue in Conzelmann (1996, *J. Gen. Virol.* 77:381-389; copy enclosed) which points out that the "observed rescue efficiency was low and was estimated on the average to occur in one in 10^7 cells," (see Conzelmann, 1996, *ibid,* p. 386, left column). The length of the genome of rabies virus is about 12 kb, while that of paramyxovirus is about 15 kb or more. Conzelmann asserted, "in rhabdovirus systems, it appeared that each additional kb of RNA in the model genomes resulted in approximately 10-fold drop in recovery rate," (see Conzelmann, 1996, *ibid*, p. 385, right column). Therefore, it can be estimated that the rescue efficiency of a recombinant virus having a 15 kb-long genome, which is 3kb longer that that of wild-type rabies virus, would be as low as one in 10^{10} cells. With such a low efficiency, it is not reasonable to expect a recovery of a recombinant virus having the genome spanning 15 kb or more by a culture system as that used in Conzelmann references. Accordingly, the Conzelmann '085 application and the Conzelmann '086 patent both fail to teach the production of recombinant paramyxovirus having a foreign gene.

Sendai Virus is a Highly Useful Gene Transfer Vector and Is Non-Pathogenic to Humans

The Conzelmann references also fail to teach the engineering not only of paramyxoviruses having a foreign gene positioned downstream of a viral gene, but also Sendai virus. Rabies virus causes central nervous system disorders, severe spasms, and bleeding in humans, and is lethal once the patient becomes symptomatic. In contrast, Sendai virus is not harmful for humans, although it causes pneumonia in mice. For example, the intranasal administration of wild type Sendai virus to primates does not result in severe virulence (see, Hurwitz *et al.*, 1997, *Vaccine* 15: 533-540; copy enclosed). Also, because the infectivity of wild-type Sendai virus is activated in the presence of trypsin-type proteases, continued propagation of Sendai virus occurs only within limited tissues such as bronchial epithelia *in vivo*, and thus, is highly safe. Sendai virus is therefore an important medical gene therapy vector for humans. Indeed, Applicants and others have studied recombinant Sendai virus vectors for several years, and demonstrated its potential for gene therapy. For example, in surgical therapy for vascular disorders such as ischemic limbs, a Sendai virus vector carrying an angiogenic gene showed dramatically superior gene-transfer efficiency compared to other vectors, and exerted its therapeutic effects (reviewed in Yonemitsu *et al.*, 2002, *Surgery* 131:S261-268; copy enclosed).

Moreover, when using vectors carrying foreign genes, it is extremely vital that the expression of the foreign gene can be regulated. For example, genes encoding cytotoxic products, and those encoding signaling molecules that cause a significant biological effect even at low level of expression, have the danger of exerting cytotoxic effects when expressed at high levels within cells. Therefore, vectors that express a foreign gene at a restricted level are needed. From this perspective, Sendai virus vectors, whose foreign gene expression level can be regulated and which are not toxic to primates are particularly advantageous.

Conzelmann Fails to Predict Regulated Gene Expression Using Paramyxovirus

The Conzelmann references further lack any indication that rabies virus vectors provide a basis for manipulating paramyxovirus vectors to regulate gene expression of a foreign gene. Conzelmann *et al.* describe only that a foreign gene was inserted into the pseudogene (Ψ) region of the rabies virus and expressed. Therefore, Applicants note that not only have Conzelmann *et al.* failed to produce recombinant viruses using a virus other than the rabies virus, Conzelmann has not even prepared virus vectors having a foreign gene at a site *other than* the pseudogene of the rabies virus. In contrast, Applicants have constructed several different Sendai virus vectors having a foreign gene positioned downstream of a viral gene, and demonstrated that such vectors could not only replicate but also express the foreign protein at varying levels depending on the insertion site of the foreign gene in the paramyxovirus genome.

Furthermore, the pseudogene region, into which a foreign gene was inserted in the rabies virus by Conzelmann *et al.*, is located between the G and L open reading frames and is transcribed as a part of the G cistron mRNA (G/Ψ mRNA). The pseudogene region is characteristic of the rabies virus and other members of the lyssavirus genus, and is conserved in all members of the lyssaviruses analyzed so far (see, Schnell *et al.*, 1994, *EMBO J.* 13: 4195-4203, at page 4197, right column). The Conzelmann references showed that a foreign gene can be inserted into the pseudogene region of the rabies virus, which consists of a long non-coding region spanning approximately 0.4 kb, but have not disclosed any viruses having a foreign gene inserted into a region other than the pseudogene region. In contrast to the rabies virus, paramyxoviruses such as the Sendai virus do not have a pseudogene region, and in fact, non-coding regions are limited to a minimum. In a publication after filing his patent applications, Conzelmann stated: "The RV (rabies virus) Ψ region represents the most plastic region of all non-segmented negative-stranded RNA virus genomes and apparently has the disposition to accept insertions of additional genes (emphasis added)" (see, Schnell *et al.*, 1994, *EMBO J.* 13:

4195-4203, at page 4201, left column; copy enclosed). In particular, Conzelmann has targeted the pseudogene region as an exceptional region seen in lyssavirus genomes, and it is not scientifically reasonable to apply this principle to paramyxoviruses. When a foreign gene is inserted into less plastic regions such as the non-coding regions of paramyxoviruses, the Conzelmann teaching fails to predict whether or not viral genes would be expressed normally and exert their functions, whether or not viral propagation would not be disturbed, and whether or not the recombinant virus is capable of expressing the inserted foreign gene.

Moreover, because of Conzelmann's entirely different approach to expressing foreign genes using rabies virus, the Conzelmann references also fail to suggest or provide motivation for any method of positioning a foreign gene downstream of a viral gene as a means of regulating gene expression. Indeed, Conzelmann does not indicate that paramyxoviruses of any sort should be exploited and does not discuss the use of where to place a foreign gene for such regulated gene expression. Conzelmann *et al.* do not teach how to predict the expression level of a foreign gene inserted between each of the Sendai viral genes. This ability to regulate the expression of a foreign gene using a paramyxovirus represents a significant advance in the gene therapy field where regulation of gene expression is vital. Nowhere do the Conzelmann references disclose, suggest, or provide the guidance or the motivation for engineering such a vector.

In contrast, Applicants have discovered not only that a foreign gene is expressed when positioned downstream of a paramyxovirus gene, but also that the level of gene expression is dependent upon placement of the foreign gene within the paramyxovirus genome. Applicants' invention enables regulated expression of a foreign gene. Wild type Sendai virus has six viral genes, NP, P, M, F, HN, and L counting in order from 3' to 5' in the genomic RNA. The instant invention discloses in detail the relative expression level of a foreign gene inserted between each of the viral genes (see, for example, Fig. 5 of the specification). For example, since the expression level differs nearly 10 fold when the foreign gene is inserted before the NP gene or after the HN gene, one can modulate the

relative expression level in the range of 1 to 10 by appropriately choosing the site of insertion. Moreover, the relative expression level of the foreign gene when it is inserted into each of these sites can be predicted beforehand simply by referring to Fig. 5. As further evidence of the significance of the invention, Applicants direct the Examiner's attention to the accompanying Declaration of Akihiro Iida, where additional evidence is provided demonstrating that the expression of a foreign gene is dependent upon its placement in within the paramyxovirus genome.

In short then, it would not have been obvious to one skilled in the art, based on the teachings of the Conzelmann references, to generate the claimed vectors or to develop the claimed methods.

CONCLUSION

Applicants submit that the claims are in condition for allowance, and such action is respectfully requested.

Applicants note that the Office action was mailed to the incorrect address. Effective immediately, please address all communication in this application to:

Paul T. Clark
Clark & Elbing LLP
101 Federal Street
Boston, MA 02110

Enclosed is a petition to extend the period for replying for three months, to and including June 4, 2002.

Also enclosed is a check for $218.00 to cover the newly added claims.

If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 4 June 2002

James D. DeCamp
Reg. No. 43,580

Clark & Elbing LLP
101 Federal Street
Boston, MA 02110-2214
Telephone: 617-428-0200
Facsimile: 617-428-7045